REMARKS

This paper is being filed in response to the Office Action mailed May 28, 2004. Claims 1 to 83 are under consideration in the application. Claims 2, 11, 12, 14, 58 and 59, have been cancelled herein without prejudice. Applicants maintain the right to prosecute the cancelled claims in any related application claiming the benefit of priority of the subject application. Accordingly, upon entry of the Response, claims 1, 3 to 10, 13, 15 to 57 and 60 to 83 are under consideration.

Regarding Sequence Compliance

Allegedly the application is not in compliance with the requirements for applications having sequences. In particular, SEQ ID NO:37 has 116 amino acids but, allegedly none of the sequences of the application have a length greater than 113 amino acids, and a SEQ ID NO does not appear to have been assigned to antibody 1A6

In Figure 3, the mouse VH domain sequence has 116 amino acids, not 113. The reason for the apparent discrepancy between the amino acids actually present and the numbering of the amino acids in the figure is that the residues indicated as "a," "b" and "c" (after residue 81) are NOT counted in the amino acid numbering, so although there only appear to be 113 amino acids indicated in Figure 3, there are actually 116 residues for this mouse VH domain sequence.

As to a SEQ ID NO for antibody denoted 1A6, SEQ ID NO:37 and 38 of the Sequence Listing correspond to VH and VL domain sequence of 1A6, respectively. The description of Figures 1 and 3 in the specification has been amended to indicate the SEQ ID NOs for the recited sequences. The SEQ ID NOs added to the specification are identified in the previously filed Sequence Listing file. Accordingly, no substitute Sequence Listing is required.

Regarding the Amendments to the Drawings and Specification

In response to the Examiner's request for corrected drawings, submitted herewith are "Replacement Sheets" for Figures 3 and 5. Figures 3 and 5 Replacement Sheets reflect the abbreviations for the exemplary antibodies, namely Hum A, Hum B, Hum C, Hum D, Hum F, Hum H and Hum I used in the specification. Support for the Figure 3 Replacement Sheet can be found, for example, at page 5, lines 3-6, which discloses that the amino acid sequence shown in Figure 3 is humanized 1A6, "Hum B." Support for the Figure 5 Replacement Sheet can be

found, for example, at page 5, lines 9-11, which discloses that the protection data shown in Figure 5 is for "HumA, HumB, HumC, HumD, HumF, HumH and Hum I." Support for the Figure 5 Replacement Sheet can also be found, for example, in Figure 3, which discloses Hum19 amino acid sequence, which corresponds to Hum B; at Table 4 (pages 47-48), which discloses EC 50 of exemplary antibodies in Figure 5; at page 48, lines 26-30, which discloses protection efficacy of Hum 19, as shown in Figure 5; and at page 48, line 31, which discloses that in vitro protection correlated well with antibody binding affinity (Table 4). Thus, Figures 3 and 5 Replacement Sheets do not add new matter and entry thereof is respectfully requested.

The amendments to the specification were made to address various informalities or are supported by the specification. In particular, the amendments at pages 2 to 4, 13 and 17 were made in order to correct various typographical errors. The amendments to the Figure 1 and 3 descriptions were made in order to insert SEQ ID NOs. The amendments to Table 4 (page 47 to 48) were made in order to use abbreviations consistent with the abbreviations used in the specification for exemplary antibodies listed, namely Hum A, Hum B, Hum C, Hum D, Hum F, Hum H and Hum I. The amendments at page 48, lines 26-30, were made to correctly reference Table 4 and Figure 5, and to use a consistent abbreviation for exemplary antibody, HumB. Support for the amendment to substitute HumB for Hum19 is as set forth above. Thus, the amendments to the specification do not add new matter and entry thereof is respectfully requested.

Regarding the Claim Amendments

The claim amendments were made to address informalities or are supported throughout the specification. In particular, the amendment to claim 1 to recite "comprising" was made in view of the prior amendment to originally filed claim 1 deleting reference to non-elected antibodies recited in a markush group. The amendment to claim 1 to recite "subsequence thereof" is supported, for example, by originally filed claims 2 and 3. The amendment to claim 1 to recite that "a variable framework region of the humanized antibody has at least one non-human amino acid substituted with a human amino acid" is supported, for example, at page 5, lines 15-19; and page 34, line 16, to page 36, line 18. The amendments to claims 5 and 22 to recite similar language are also supported, for example, at page 5, lines 15-19; and page 34, line 16, to page 36, line 18. The amendments to claim 2 and 15 were made in order to conform

the claim language with amended claim 1 and to improve clarity. The amendment to various claims to delete "the" and insert "a" was made in order to address an alleged lack of adequate antecedent basis. The amendment to claims 6 to 10 was made in order to improve antecedent basis for the non-humanized antibody, 1A6 and is supported, for example, at page 5, lines 16-19; page 6, lines 2-7, which discloses that affinity for humanized antibody can "range from greater or less affinity for the antigen than either donor or recombinant antibody," and in particular aspects, "greater than parental antibody;" and page 7, lines 9-19, which discloses the type and source of various donor antibody sequences, including antibody 1A6. The amendment to claims 4, 13, 23 and 48 to delete certain language was made in order to eliminate redundant claim language. The amendment to claims 3, 15 and 24 to insert "Fv" is supported, for example, at page 10, lines 8-9. The amendment to claim 22 to delete certain language was made in view of the prior amendment to originally filed claim 1 deleting reference to non-elected antibodies recited in a markush group. The amendment to claim 28 to delete certain language is supported, for example, at page 17, lines 4-6. The amendment to claims 31 and 33 to depend from claims 30 and 32, respectively, was made in order to correct an obvious typographical error. The amendment to claims 47, 50 and 55 were made to correct a typographical error. The amendment to claims 52 and 57 to recite "years" was made in order to correct an obvious error and is supported, for example, at page 33, lines 8-10, which discloses that human subjects include "children, for example, newborns and older children, for example, between the ages of 1 and 5, 5 and 10 and 10 and 18." The amendment to claim 53 to recite "a symptom" was made in order to improve clarity. The amendment to claims 60 to 62 to insert the terms "framework region" and "human" were necessitated by the amendment to claim 4, and are supported, for example, by originally filed claim 4, and as set forth above for the amendment to claims 1, 5 and 22. The amendment to claims 63 to 72 to insert the term "determining" is supported, for example at page 5, lines 14-15; and page 7, lines 20-21. The amendments to claims 63 to 83 to insert either or both of the terms "substituted" or "unsubstituted" were made in order to provide greater clarity to the referenced antibodies. The amendment to claim 73 to recite "greater than" is supported, for example, at page 6, lines 2-3. Thus, the amendments to the claims do not add new matter and entry thereof is respectfully requested.

Regarding the Copies of References filed on PTO-1449

In the previous Response filed May 5, 2003, Applicants submitted for the Examiner's consideration a copy of a reference, denoted PPR in the previously filed PTO-1449 form. Applicants respectfully requested that the Examiner consider reference PPR. However, the Office Action mailed May 28, 2004, does not indicate that reference PPR was considered. Applicants therefore reiterate the request that the Examiner consider reference PPR in light of the claims under consideration.

I. OBJECTION TO THE DISCLOSURE

The disclosure stands objected to due to various informalities. Applicants are requested to update the status of related applications, to correct various typographical errors and to clarify certain text in the application.

The status of the related applications is current and, therefore, no updating is necessary. The specification has been amended as set forth above to correct typographical errors, as requested by the Examiner. The specification has been amended to use consistent abbreviations for exemplary antibodies Hum A, Hum B, Hum C, Hum D, Hum F, Hum H and Hum I, and to reference Table 4 and Figure 5. As to the request at page 4 of the Office Action, in particular, to "specify the residues in which the short amino acids, ADSVK and DPKVQ, correspond," Applicants do not understand the action requested, nor the grounds for the request. Accordingly, clarification of this issue is respectfully requested.

II. OBJECTION TO CLAIMS

Claims 1 to 3, 22, 47, 50, 55 and 63 to 72 stand objected to due to various informalities. Applicants are requested to correct various typographical and grammatical errors in the claims.

As set forth above, the claims have been amended in order to address each of the informalities. In particular, the spelling and grammatical errors have been corrected. In addition, claims 63 to 72 have been amended to insert the term "determining." In view of the amendment to claim 1, claims 2 and 3 are not in improper dependent form. As to the Examiner's request to replace the term "subsequence" with the term "fragment," Applicants agree with the Examiner that the terms are interchangeable. Thus, such an amendment would not have been made for reasons related to patentability. However, Applicants respectfully point out that

because there is a remote possibility that such an amendment may be construed to have been made for reasons related to patentability, that Applicants be allowed to keep the term "subsequence" in the claims. Because the skilled artisan would know the meaning of the term "subsequence," in view of the specification, for example, at page 10, lines 3-7, this term does not render the claims indefinite under 35 U.S.C. §112, second paragraph. In view of the amendments and foregoing remarks, Applicants respectfully request that the objection to the claims be withdrawn.

III. REJECTIONS UNDER 35 U.S.C. §112

The rejection of claims 1 to 83 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is respectfully traversed. The grounds for rejection relate to allegedly unclear claim language, and lack of adequate antecedent basis for various terms.

The claims, as originally filed, are clear and definite as written. Nevertheless, claim 14 has been cancelled herein without prejudice, and the claims have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

Claim 1 has been amended to recite "a humanized antibody that binds ICAM-1, comprising SEQ ID NO:5 and 7 (HumB), or a subsequence thereof." In view of the amendment, the skilled artisan would know that the claimed antibody comprises a combination of SEQ ID NO:5 and 7, or a subsequence thereof. Claims 2, 4 to 10, 22, 28, 31 and 33 have been amended to correct for the alleged lack of antecedent basis. Claims 13, 15 to 17 and 21 have been amended to recite "humanized" antibody in order to more clearly indicated the referenced antibody. Claims 52 and 57, as amended, recite "years," which corrects an obvious error.

Claim 53, as amended, recites " a symptom." As to symptoms encompassed by claim 53, the specification discloses, for HRV, for example, fever, congestion, cough, nasal drip and sore throat. Additional symptoms are known in the art and include, for example, red eyes. As to how a decrease in symptoms of the common cold could occur in a subject having symptoms of a cold, the specification further discloses that administering prior to or immediately following development of symptoms may lessen their severity. Thus, in view of the guidance in the specification, the skilled artisan would know that a subject having a cold treated in accordance with the invention could undergo a decrease in fever duration, as measured over time (e.g., days)

or fever severity, as measured by the subject's temperature; congestion, as measured by the degree of nasal or sinus blockage or sputum production; cough, nasal drip and sore throat, as measured by their frequency or intensity, for example. Thus, the specification discloses a variety of symptoms and the skilled artisan would know how to measure a decrease in such symptoms. As to how a decrease in symptoms of the common cold could occur in a subject not having a symptom of a cold, claim 53 recites that the subject has a cold. Furthermore, the skilled artisan would know that treating a subject prior to development of symptoms may delay onset or reduce their severity or duration because the common cold is known to cause one or more symptoms, as disclosed in the specification and known in the art (e.g., red eyes). Thus, even if a subject having a cold does not exhibit at least one symptom, even if the symptom was mild, because the skilled artisan knows that HRV is an etiological agent that causes a cold, and knows the symptoms associated with the cold, clearly the skilled artisan could ascertain the status of a causative agent or a symptom of the common cold. As to how a decrease in symptoms of the common cold could occur in a subject not having a cold, claim 53 recites "a subject having a cold." Consequently, Applicants need not address this issue.

Claims 63 to 83, which depend from claim 4, as amended, are clear and definite as to the nature of the recited antibodies. As to how humanized antibody made up of CDRs of 1A6 can have better affinity for ICAM-1 than a humanized antibody having the CDR regions of 1A6, humanized antibodies of claims 63 to 83 have one or more amino acid substitutions in the variable framework region based upon a human consensus variable framework region sequence, which provides greater binding affinity than unsubstituted humanized antibody. Consequently, claims 63 to 83 directed to humanized antibodies having a substitution and which has greater binding affinity for ICAM-1, as compared to unsubstituted humanized antibody made up of CDRs of 1A6. Accordingly, amended claims 63 to 83 are clear and definite.

As to the recitation of "100 fold or greater" in claims 72 and 83, the plain meaning of this language in the context of the claims indicates that antibody binding affinity for ICAM-1 is 100-fold or greater than the comparison (unsubstituted) antibody. Furthermore, the specification discloses that affinity of humanized antibody can be greater than parental or donor antibody. Accordingly, in view of the plain meaning of the claim language and the specification, the skilled artisan would understand that "100-fold or greater" means that the ICAM-1 binding affinity is increased as compared to the other (unsubstituted) antibody, by 100-fold or greater.

In view of the foregoing, the claims are clear and definite. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph, be withdrawn.

The rejection of various claims under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description, is respectfully traversed. The grounds for rejection for the claims at issue are discussed below.

Claims 5 and 73 allegedly lack support for the recitation of "at least equivalent." Applicants traverse. In this regard, Applicants first wish to point out that "the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims." *In re Wertheim*, 541 F.2d 257, 263 (C.C.P.A. 1976). Here, the PTO has not provided any reason why the skilled artisan would not recognize that the specification discloses humanized antibodies having equivalent affinity as mouse monoclonal antibody denoted as 1A6. Furthermore, the Examiner acknowledges that the specification teaches antibodies having "greater or less affinity." Throughout the specification, antibodies having greater or less affinity than mouse monoclonal antibody denoted as 1A6 are taught. Moreover, the specification, at page 6, lines 3-5, discloses in relevant part, that antibody "affinities *range* from greater or less affinity for the antigen than the donor or recombinant antibody." [*Emphasis added*] The common meaning of "range" is an area within which something exists. In *Wertheim*, the court held that because the specification disclosed a range of 25%-60% that a limitation to "between 35% and 60%" met the description requirement. [see, M.P.E.P. §2163.06] Thus, in view of the fact that the area within greater or less affinity includes equivalent affinity, clearly humanized antibodies having "equivalent" affinity as mouse monoclonal antibody denoted as 1A6 are disclosed in the specification. In any event, claims 5 and 73 have been amended to recite "greater than." Accordingly, the ground for rejection is moot.

Claims 71 and 82 allegedly lack support for the recitation of "50 to 100." Applicants traverse. In this regard, the PTO acknowledges that the specification teaches 20-to 100-fold. Applicants again respectfully again direct the Examiner's attention to *In re Wertheim*, in which the court held that because the specification disclosed a range of 25%-60% that a limitation to

"between 35% and 60%" met the description requirement. [see, M.P.E.P. §2163.06] As such, the ground for rejection is improper and must be withdrawn.

Claims 2 to 3 allegedly lack an adequate written description for fragments of HumB. Allegedly, the fragments need not possess any particular distinguishing feature, biologic activity or conserved structure. Claim 2 has been cancelled herein without prejudice rendering the rejection moot. Claim 1 has been amended to include subject matter of cancelled claim 2. Accordingly, Applicants traverse.

Applicants respectfully remind the Patent Office that, "[a] specification may, within the meaning of 35 U.S.C. §112¶1, contain a written description of a broadly claimed invention without describing all species that claim encompasses." *Utter v. Hiraga,* 845 F.2d 993, 998 (Fed. Cir. 1988). In addition, the courts have repeatedly held that "every species in a genus need not be described in order that a genus meet the written description requirement." *Reagents of the Univ. Calif. v. Eli Lilly*, 119 F.3d 1559, 1568 (Fed. Cir. 1997). Nor are Applicants "required to disclose every species encompassed by their claims, even in an unpredictable art." *In re Angstadt*, 537 F.2d, 498, 502-503 (C.C.P.A. 1976). Thus, clearly a description of all HumB subsequences need not be described in order to satisfy the written description requirement under 35 U.S.C. §112, first paragraph.

Here, HumB subsequences of claims 1 and 3 are defined structurally and functionally. First, HumB subsequences are defined by sequence, namely fragments of SEQ ID NO:5 or 7. Second, HumB subsequences of claims 1 and 3 have a defined activity, namely binding to ICAM-1 (see claim 1). Thus, contrary to the Office Action, the claimed HumB subsequences have a defined structure based upon SEQ ID NO:5 and 7, and a defined function, binding to ICAM-1.

As to conserved structure among HumB subsequences, claim 3 is directed to five types of antibody subsequences, namely single chain, Fv, Fab, Fab' and $(Fab)_2$, each of which have defined structures known to the skilled artisan. Additional subsequences are known in the art (for example, Fd, a fragment of Fab, VH + CH1). Thus, at least six specific examples of HumB antibody subsequences would be known to the skilled artisan.

As to additional HumB antibody subsequences of SEQ ID NO:5 and 7, given the guidance in the specification and knowledge in the art regarding antibody structure and function,

the skilled artisan would know of a variety of HumB subsequences that retain ICAM-1 binding activity. For example, the skilled artisan would know sequence regions that contribute to antigen binding, such as complemtarity determining regions (CDRs) and framework regions (FRs). The specification discloses CDR sequences of HumB V_H and V_L domains, and based upon their location, the skilled artisan would also know the location of the FRs. In view of this knowledge, the skilled artisan would therefore know that HumB subsequences having one or a few amino acids deleted outside the CDRs or FRs or from the amino- or carboxy-terminus, would retain at least partial ICAM-1 binding affinity.

Finally, satisfying the written description requirement 35 U.S.C. §112, first paragraph, is a factual determination, and the claims are factually distinguishable from the cited case law, namely *Fiers v. Revel*, 984 F2d 1164 (Fed. Cir. 1993); *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 927 F.2d 1200 (Fed. Cir. 1991); and *Fiddes v. Baird*, 30 USPQ2d 1481 (B.P.A.I. 1993). In each of these three cases, the court or B.P.A.I. held that the failure to disclose any sequence or only one sequence was insufficient to describe a genus of sequences. In *Fiers*, a priority claim based upon an application that failed to disclose even one β-interferon sequence was rejected, the court stating "[w]e also reject Fiers' argument that the existence of a workable method for preparing a DNA establishes conception of that material." As to Revel's claim to priority, the court stated in *Fiers* that "[a] bare reference to a DNA with a statement that it can be obtained by reverse transcription is not a description." *Id.* In *Amgen*, although the holding was based upon a lack of enablement, the court stated that to conceive a gene sequence, "[i]t is not sufficient to define it solely by its principal biological property." *Amgen* at 1205. In *Fiddes*, the Board held that because the patent failed to teach any FGF, other than bovine pituitary FGF, which actually was a postulated sequence that turned out to be incorrect when the naturally occurring bFGF sequence was subsequently isolated, that a written description for the broad class of mammalian FGFs was not provided. *Fiddes* at 1483-4. In contrast to these three cases, as discussed above, the specification discloses five particular HumB subsequences. Furthermore, additional HumB subsequences, such as Fd, would be known to the skilled artisan. Moreover, in view of the knowledge in the art regarding antibody structure and function, the skilled artisan would have known even additional HumB subsequences.

In sum, in view of the fact that the HumB subsequences of claims 1 and 3 are defined both structurally, by sequence; and functionally, by activity; and further in view of the fact that

additional HumB subsequences would be known to the skilled artisan, an adequate written description for HumB subsequences of claims 1 and 3 is provided. As such, the grounds for rejection are improper and must be withdrawn.

Claims 4 and 58 to 83 allegedly lack an adequate written description for HumB amino acid substitutions. Allegedly, the disclosure of six different antibodies each having greater binding affinity than humanized antibody having CDRs of mouse monoclonal denoted as 1A6 is insufficient to adequately describe a genus of antibodies claimed. The grounds are based upon the alleged absence of any antibody having greater binding affinity than mouse 1A6.

Claims 4 and 58 to 83 are adequately enabled. Applicants respectfully point out that the data disclosed in Table 4 has been misunderstood by the Patent Office. In particular, the smaller the numerical value for K_D (the dissociation constant) the **greater** the affinity. The K_D (M) of HumA is 1.50×10^{-7}, and for 1A6 is 1.18×10^{-6}. The affinity difference is therefore greater than 10-fold. Consequently, the affinity of all six of the exemplified antibodies, HumA, HumB, HumC, HumD, HumF, HumH and HumI, for ICAM-1 is at least 10-fold greater than mouse antibody1A6 (pages 47-48, Table 4). Thus, all six antibodies have the requisite affinity for ICAM-1.

Turning to the grounds for rejection at page 14, second paragraph, allegedly that "the number of antibodies that are taught in the specification does not adequately enable nor does it sufficiently represent the genus of antibody that can be derived from HumB," the specification exemplifies six different antibodies, each of which have the requisite ICAM-1 binding affinity. Additional antibodies can be produced without undue experimentation in view of the guidance in the specification and knowledge in the art. For example, the specification discloses that following grafting of a non-human murine CDR into a human framework region, that one or more of the murine sequences can be mutated to human sequences, such as mutating a non-human FR amino acid to a human amino acid (page 5, lines 14-19). The specification also discloses that human antibody sequence regions can be used to produce humanized antibodies, and can be based, for example, on a human consensus sequence (page 6, line 8, to page 77, line 8). The specification further discloses detailed methods for producing humanized antibodies, including various parameters to consider for selecting amino acid substitutions (page 34, line 9, to page 36, line 18). The specification additionally discloses a variety of sources for donor

CDRs (page 7, lines 9-19) and amino acid substitutions that can be made including for example, conservative substitutions (page 14, line 29, to page 15, line 7). Binding affinity to ICAM-1 can be measured by routine methods disclosed in the specification or known in the art for example, ELISA, immunoprecipitation, etc. (page 47, lines 6-19).

Thus, in view of the guidance in the specification, additional humanized antibodies based upon HumB that have at least one non-human amino acid substituted with a human amino acid which bind ICAM-1, as in claims 4 and 58 to 83, can be produced without undue experimentation.

Turning to the ground for rejection, at page 14, second paragraph, page 16, second paragraph, and page 19, second paragraph, allegedly that Applicant has not provided "the specific activities that the newly derived antibody must possess," Applicants respectfully point out that the claimed antibodies bind to ICAM-1, under appropriate conditions for the binding to occur. In this regard, the Examiner will appreciate that even an antibody having a high binding affinity for ICAM-1 will not bind under all conditions because antibody-antigen binding is non-covalent. Such binding therefore will not occur for example, when there are conditions that disrupt such binding, such as large quantities of detergent or extreme heat. Although it is alleged in the Office Action that "capable" is a conditional term and, that "capable of binding to ICAM-1 do not necessarily mean the newly derived antibodies bind to ICAM-1," Applicants respectfully point out that here the meaning of the phrases, namely "capable of binding to ICAM-1" and "binding to ICAM-1" are identical. In any event, the claims have been amended to delete the term "capable." As such, this ground for rejection is moot. Applicants stress that the amendment to delete "capable" was made for reasons unrelated to patentability, since antibody binding to ICAM-1 of the originally filed claims is identical to antibody binding to ICAM-1 after entry of the amendment deleting the term "capable" from the claims.

Turning to the ground for rejection at page 15, second paragraph, page 17, first paragraph, and page 18, second paragraph, allegedly that Applicant has not made substitutions within the CDR regions of the humanized antibodies, the claims, as amended, indicate that the amino acid substitution of non-human to human is located in a variable framework region. As such, this ground for rejection is moot.

Turning to the grounds for rejection at pages 21 to 24, as applied to claims 5 to 57, claim 12 has been cancelled without prejudice, and claims 5 and 36, as amended, recite that the

pathogen is "HRV." Claim 15, as amended, depends from claim 1. Thus, the grounds for rejection based upon an alleged lack of enablement of "any pathogen," are moot. The rejection will therefore be addressed with respect to the amended claims.

The claims, as amended, are adequately enabled. Turning to the protective efficacy against HRV data, the specification discloses that the claimed antibodies are effective at protecting cells from HRV infection. In particular, for example, the specification discloses that each of the humanized antibodies protect cells from HRV (Pages 47-48, Table 4; page 48, lines 26-31; and Figure 5). Thus, the data indicate that the claimed antibodies can protect against HRV infection.

It is unclear upon what basis the Patent Office questions the protection data disclosed in the specification (see Office Action, paragraph bridging pages 21 and 22). In this regard, the assay used for determining HRV protection is a routine crystal violet cell viability assay. In this assay protection from HRV is proportional to the amount of crystal violet, which accumulates in viable cells and is measured by absorbance, as disclosed in the specification. The greater the amount of crystal violet, the greater the number of viable cells present and, hence, the greater the protection provided by the antibodies against HRV.

Turning to the ground for rejection at page 22, and HeLa cells, HRV infects HeLa cells, as discussed above. Furthermore, studies of HRV infection with HeLa cells are recognized in the art as predictive of therapies for HRV infection *in vivo*. In this regard, HeLa cell based HRV infectivity studies have been used to produce data to support FDA approval of clinical trials for various human HRV therapies. For example, Exhibit A, a publication by Kaiser *et al.* (Antiviral Res. 47:215 (2000)), reports HeLa cell studies with AG7088, which was effective against HRV infection of HeLa cells, as determined by increased cell viability (see Exhibit A, abstract). AG7088, also known as "Rupritrivir" subsequently proceeded into human clinical trials, and was found to have anti-HRV activity, the results of which are reported in Exhibit B, a publication by Hayden *et al.* (Antimicrob Agents Chemother. 47:3907 (2003); see, for example, abstract). HeLa cells have also been used to measure anti-HRV activity of other HRV therapies that subsequently proceeded into clinical trials (see, for example, Colonno *et al.*, J. Virol. 57:7 (1986)); and Hayden *et al.*, Antiviral Res. 9:233 (1988)). Thus, HeLa cells are recognized in the art as predictive of therapies for HRV infection *in vivo*.

IV. REJECTIONS UNDER 35 U.S.C. §103(a)

The rejection of claims 1 to 3 and 34 to 39 under 35 U.S.C. §103(a) as allegedly unpatentable over Colonno *et al.* (EP 459577 A2) in view of two Padlan publications (Mol. Immunol. 28:489 (1991); and Mol. Immunol. 31:169 (1994)) is respectfully traversed. Colonno *et al.* allegedly report "mouse monoclonal antibody 1A6...demonstrated to have binding affinity for ICAM-1." The Examiner acknowledges that Colonno *et al.* "does not teach a humanized version of mouse monoclonal antibody 1A6." Allegedly, Padlan describe human consensus sequences. Allegedly, it would have been obvious to combine the references to produce a humanized version of 1A6, and motivation is in using the antibody for "therapeutic purposes in human."

Originally filed claims 1 to 3 and 34 to 39 would not have been obvious in view of Colonno *et al.* (EP 0459577 A2) or the Padlan publications (Mol. Immunol. 28:489 (1991); and Mol. Immunol. 31:169 (1994), referred to hereinafter as "Padlan 1991" and "Padlan 1994") alone, or in combination. Nevertheless, solely in order to expedite prosecution of the application and without acquiescing to the propriety of the rejection, claims 2 has been cancelled herein without prejudice, and the claims have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

In order for a rejection to be proper under 35 U.S.C. §103, *inter alia*, there must have been 1) a motivation to combine the references at the time of the invention; 2) the combination of references must teach or suggest each and every element of the claimed invention; and 3) there must have been a reasonable expectation of success at the time of the invention. Both the teaching or suggestion to make the claimed combination and the reasonable expectation of success must be found in the prior art, not in Applicants' disclosure. See, e.g., *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991); and *In re O'Farrell*, 853 F.2d 894, 903-904 (Fed. Cir. 1988). Here, *inter alia*, the combination of Colonno *et al.*, Padlan 1991 and Padlan 1994, 1) fails to teach or suggest each and every element of the claimed; 2) fails to provide a motivation to produce the claimed antibodies; and 3) fails to provide a reasonable expectation of success of producing the claimed compositions at the time of the invention. Furthermore, the art must be considered in its entirety, including portions that would lead away from the invention, and Padlan 1991 and Padlan 1994 each teach away from producing the claimed antibodies. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983).

The amended claims, recite, *inter alia*, that the humanized antibody that binds ICAM-1 has a variable framework region in which at least one non-human amino acid has been substituted with a human amino acid. Claims 5 and 22 require that that the human amino acid be of a human consensus variable framework region sequence. However, as discussed in the record, Colonno *et al.* fail to teach or suggest a humanized antibody in which a variable framework region of the humanized antibody has had one or more non-human amino acids substituted with a human amino acid, let alone a human amino acid of a human consensus variable framework region sequence. In this regard, Colonno *et al.* fail to teach or suggest any human framework region sequence, let alone a sequence having one or more amino acids of a human consensus variable framework region sequence.

The Padlan 1991 publication reports humanizing antibodies to reduce immunogenicity of variable domains. The Padlan 1994 publication is a review article of antibody structures. However, neither Padlan publication teaches or suggests a humanized antibody in which a variable framework region of the humanized antibody has had one or more non-human amino acids substituted with a human amino acid, let alone teach or suggest a human amino acid of a human consensus variable framework region sequence. Consequently, the combination of Colonno *et al.* and the Padlan publications fail to teach or suggest each and every element of the claimed humanized antibodies.

Furthermore, both Padlan publications teach retaining donor-antibody framework sequences in order to preserve antigen binding. Any amino acid substitutions to the humanized antibodies should be made from a human amino acid to a non-human amino acid. This selection is based upon the identity of the amino acid at that position in the non-human donor sequence.

In Padlan 1991, for example, the author states that antigenicity can be reduced "through replacement of the exposed residues in the framework regions which differ from those usually found in host antibodies." (abstract) The author provides greater details on producing humanized antibodies, stating that "one would retain (a) the CDRs, (b) the residues in the immediate neighborhood of the CDRs." (page 495, first column) Amino acid positions in which the mouse and human sequences differ are recommended to be kept mouse (see, for example, page 495, second column, third paragraph) In the conclusion, the author discusses prior work by Winter, who grafts non-human CDRs onto human frameworks, stating that "ligand affinities of modified molecules have been found to be drastically reduced so that it was deemed necessary

to replace some framework residues with in attempts to recover the binding properties of the original antibodies." (page 497, first column, third paragraph) Consequently, Padlan 1991, which teaches retaining donor-antibody (non-human) framework sequences in order to preserve antigen binding, teaches away from producing the claimed humanized antibodies.

Padlan 1994 is analogous to Padlan 1991 with regard to teaching the importance of retaining donor-antibody (non-human) framework sequences in the humanized antibodies in order to preserve antigen binding activity. For example, in Padlan 1994, the author states that "some framework residues from the original antibody, those which influence the structure of the combining site, also need to be preserved." (page 201, second column, first paragraph) The author then points out that all framework residues should be retained, stating "with the view towards preserving their ligand binding properties, it would probably be wise to retain all of its framework residues." (page 201, second column, last paragraph) In referring to the work of others, the author states "if during 'humanization,' not just the CDRs are transplanted, but also some of the residues immediately adjacent to the CDRs, there would be a better chance of retaining the ligand-binding properties of the original antibody." (page 204, first column, last paragraph) Consequently, Padlan 1994, which also teaches retaining donor-antibody (non-human) framework sequences in order to preserve antigen binding, also teaches away from producing the claimed humanized antibodies.

In view of the fact that both Padlan publications teach away from producing the claimed humanized antibodies, the skilled artisan would not have been motivated to produce the claimed humanized antibodies at the time of the invention. Furthermore, in view of the frequency that humanized antibodies having human framework residues had reduced affinity compared to the donor non-human antibody, as discussed in both Padlan publications, the skilled artisan would not have had any reasonable expectation that the claimed humanized antibodies, even if produced, would retain affinity for ICAM-1, let alone have equivalent or greater affinity for ICAM-1, or protective efficacy against HRV, than mouse monoclonal antibody denoted as 1A6. Consequently, the skilled artisan would not have had the requisite motivation, nor the requisite reasonable expectation of success in view of both Padlan publications.

In sum, as Colonno *et al.* and the Padlan publications fail to teach or suggest each and every element of claims 1 to 3 and 34 to 39, fail to provide the skilled artisan with the requisite motivation to produce claims 1 to 3 and 34 to 39, and fail to provide a reasonable expectation of

success at producing claims 1 to 3 and 34 to 39, these claims would not have been obvious in view of Colonno *et al.* and the Padlan publications. Accordingly, the rejection of claims 1 to 3 and 34 to 39 under 35 U.S.C. §103(a) is improper and must be withdrawn.

The rejection of claims 4, 16 to 21, 28 to 35 and 53 to 83 under 35 U.S.C. §103(a) as allegedly unpatentable over Colonno *et al.* (EP 459577 A2) in view of two Padlan publications (Mol. Immunol. 28:489 (1991); and Mol. Immunol. 31:169 (1994)) is respectfully traversed. Colonno *et al.* and the two Padlan publications, namely Padlan 1991 and Padlan 1994, have been discussed above. Allegedly, at the time of the invention, it would have been obvious to produce the claimed antibody by combining Colonno *et al.* and Padlan, and "make different amino acid substitutions within the humanized antibody....as a part of routine experimentation." [Office Action, page 27] The further grounds for rejection as to claims 16 to 21, 28 to 33, 34 and 35 can be found in the Office Action.

Originally filed claims 4, 16 to 21, 28 to 35 and 53 to 83 would not have been obvious in view of Colonno *et al.* (EP 0459577 A2) or the Padlan publications (Mol. Immunol. 28:489 (1991); and Mol. Immunol. 31:169 (1994), referred to hereinafter as "Padlan 1991" and "Padlan 1994") alone, or in combination. Nevertheless, solely in order to expedite prosecution of the application and without acquiescing to the propriety of the rejection, claims 11, 12, 14, 58 and 59 have been cancelled herein without prejudice, and the claims have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

Applicants first respectfully point out that the Patent Office has used an improper standard to determine obviousness under 35 U.S.C. §103(a). In particular, it is stated that it would have been obvious to take the humanized antibody produced and "make different amino acid substitutions within the humanized antibody...*as a part of routine experimentation.*" [Emphasis added] However, there is no objective evidence to support this conclusory statement. In this regard, the courts are clear: merely because references can be combined or modified does not render the resultant combination obvious unless the prior art suggests the desirability of the combination. *In re Mills*, 916 F.2d 680 (Fed. Cir. 1990). The use of a standard for obviousness based upon "routine experimentation" is analogous to an "obvious to try standard," or what was "well within the ordinary skill of the art," which have been repeatedly rejected by courts. *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988) (*citations omitted*); *Ex parte Levengood*, 28

U.S.P.Q. 2d 1300 (B.P.A.I. 1993). Accordingly, Applicants respectfully request that the proper standard for obviousness under 35 U.S.C. §103(a) be used.

As discussed above, neither Padlan publication teaches or suggests a humanized antibody in which a variable framework region of the humanized antibody has had one or more non-human amino acids substituted with a human amino acid, let alone teach or suggest a human amino acid of a human consensus variable framework region sequence. Consequently, the combination of Colonno *et al.* and the Padlan publications fail to teach or suggest each and every element of the claimed humanized antibodies.

Furthermore, both Padlan publications teach retaining donor-antibody (non-human) framework sequences in order to preserve antigen binding, which teaches away from producing the claimed humanized antibodies. Consequently, the skilled artisan, at the time of the invention, would not have been motivated to produce the claimed humanized antibodies. Furthermore, because humanized antibodies having human framework amino acids frequently had reduced affinity compared to donor non-human antibody, as discussed in both Padlan publications, the skilled artisan would not have had any reasonable expectation that the claimed humanized antibodies, would retain affinity for ICAM-1, let alone have equivalent or greater affinity for ICAM-1, or protective efficacy against HRV, than mouse monoclonal antibody denoted as 1A6. Consequently, the skilled artisan would not have had the requisite motivation, nor the requisite reasonable expectation of success in view of the Padlan publications.

In sum, as Colonno *et al.* and the Padlan publications fail to teach or suggest each and every element of claims 4, 16 to 21, 28 to 35 and 53 to 83, fail to provide the skilled artisan with the requisite motivation to produce claims 4, 16 to 21, 28 to 35 and 53 to 83, and fail to provide a reasonable expectation of success at producing claims 4, 16 to 21, 28 to 35 and 53 to 83, these claims would not have been obvious in view of Colonno *et al.* and the Padlan publications. Accordingly, the rejection of claims 4, 16 to 21, 28 to 35 and 53 to 83 under 35 U.S.C. §103(a) is improper and must be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 1, 3 to 10, 13, 15 to 57 and 60 to 83 clearly and patentable define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4065.

Please charge any additional fees, or make any credits, to Deposit Account No. 03-3975.

Respectfully submitted,

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Short communication

In vitro activity of pleconaril and AG7088 against selected serotypes and clinical isolates of human rhinoviruses☆

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Abstract

Background: We tested the in vitro activity of pleconaril and AG7088 against five numbered human rhinovirus (HRV) serotypes and 46 clinical HRV isolates of undefined serotype recovered from patients with common colds. Antiviral effect of pleconaril and AG7088 were assessed by cytophathic effect (CPE) inhibition assays in Ohio HeLa1 cells using microscopic and spectrophotometric methods. Both compounds were tested at concentrations of 1.0, 0.1 and 0.01 μg/ml. For the numbered HRV serotypes, the median EC_{50} value determined by the microscopic CPE inhibition was slightly better for AG7088 compared to pleconaril ($P = 0.02$) but was similar by spectrophotometric assay ($P = 0.15$). For clinical HRV isolates the median EC_{50} value determined microscopically was 0.01 μg/ml range, $< 0.01-0.04$ μg/ml) for AG7088 compared to 0.07 μg/ml (range, $< 0.01- > 1$ μg/ml) for pleconaril ($P < 0.001$). The median EC_{50} value determined by spectrophotometric assay was 0.01 μg/ml (range, $< 0.01-0.04$ μg/ml) for AG7088 compared to 0.04 μg/ml (range, $< 0.01- > 1$ μg/ml) for pleconaril ($P < 0.001$). By either the microscopic or spectrophotometric methods the median EC_{50} value of AG7088 was < 1.0 μg/ml for all isolates and was > 10.0 μg/ml of pleconaril for approximately 9% of isolates. In vitro AG7088 appeared to be more potent and to have a broader antirhinoviral spectrum than pleconaril among clinical HRV isolates. The clinical relevance of these in vitro results needs to be determined in controlled clinical trials.

Keywords: Human rhinovirus; AG7088; Pleconaril; Protease inhibitors; Capsid binding agents

1. Introduction

No effective antirhinoviral therapy is currently available for clinical use, but new antiviral agents are in development (Arruda et al., 1997; Turner et al., 1999). Pleconaril is a capsid function inhibitor which targets a conserved hydrophobic pocket of

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Exhibit A

a major viral capsid protein called VP-1. Pleconaril inhibits attachment and/or virus uncoating (Arruda et al., 1997).

AG7088 is an irreversible inhibitor of HRV 3C protease responsible for the cleavage of viral polyproteins into essential proteins (Matthews et al., 1999). The coding region of the active site is conserved among HRV serotypes, and in vitro studies have shown that AG7088 is a potent inhibitor of HRV (Matthews et al., 1994; Patick et al., 1999). AG7088 is formulated for intranasal delivery.

Little data on the in vitro activity of AG7088 and pleconaril against clinical HRV isolates are available. We tested the in vitro activity of these two compounds against five selected HRV serotypes and against clinical HRV isolates of undefined serotype recovered from patients with common colds.

2. Methods

Nasopharyngeal washes, performed in adults or children with colds between 1988 and 1998, were first inoculated onto monolayer of human embryonic lung fibroblast cells, rhinovirus was identified by acid liability testing and in the majority of cases also by RT-PCR. Rhinovirus positive nasal washes were frozen at −70°C. For the purpose of this study these specimens were thawed and inoculated onto monolayers of Ohio HeLa-I cells (Arruda et al., 1996), and frozen at −70°C when rhinovirus related cytopathic effect (CPE) of 90% was present. At the time of the antiviral assay these specimens were thawed, centrifuged and supernatant was used for the antiviral assay (Arruda et al., 1992, 1996). In addition to clinical specimens, five selected serotypes representing the two principal groups of HRV receptors (HRV-2, HRV-14, HRV-16, HRV-39 and HRV Hanks) were tested. Pleconaril and AG7088, were diluted in maintenance media at concentrations of 1.0, 0.1 and 0.01 μg/ml and a final concentration of 0.25% dimethyl sulfoxide. The molecular weight of AG7088 is 599 and the molecular weight of pleconaril 381. AG7088 was provided by Agouron Pharmaceuticals. The 50% effective inhibitory concentration (EC_{50}) of AG7088 and pleconaril were determined in CPE inhibition assay on Ohio HeLa-I cells as described (Arruda et al., 1992). For each virus isolate (inoculated at three different dilutions) and compound dilution, drugs were tested in triplicate on plates containing also cytotoxicity controls and inoculum titration (backtiters). Assays were read on inoculum for which backtiters identified a 50% tissue culture infective doses per milliliter ($TCID_{50}$/ml) between 32 and 320. Monolayers were treated sequentially with 0.05 ml of each compound solution, 0.05 ml of virus inoculum, or 0.1 ml of McCoy's media with 2% fetal bovine serum (Hyclone Labs, Pittsburgh, PA) for cell control, and incubated at 34°C in CO_2 incubator. Determination of the proportion of cells with morphologic signs of CPE (ballooning, refractiveness, granularity, shrinkage) and spectrophotometric reading (at 550 nm) were performed as previously described (Arruda et al., 1992, 1996; Ohlin et al., 1994).

The EC_{50} of the compounds was calculated using the software Dose-Effect Analysis with Microcomputers (Biosoft, Cambridge, United Kingdom). In order to calculate a median EC_{50} value we assigned arbitrary EC_{50} values of 0.005 μg/ml if the obtained EC_{50} was < 0.01 μg/ml and 2.154 μg/ml if this value was > 1.0 μg/ml. These values represent the value obtained if the next lowest drug concentration, or the next highest, would have reach 0 or 100% of inhibition respectively and are estimate of the true EC_{50} value.

Cytotoxicity was evaluated in parallel to antiviral assays on non-growing cells. In addition, inhibition of uninfected cell growth was determined in a separate experiments using the cellular protein adhering dye sulforhodamine B (Skehan et al., 1990; Rollins et al., 1993).

3. Results

3.1. Cellular toxicity

For both compounds altered cellular morphology was observed in uninfected control cells at the highest concentration tested (100 μg/ml). Slight alterations of cellular morphology were observed

at a pleconaril concentration of 10 μg/ml, but no change was observed for both compounds at concentration of 1.0 μg/ml. For growing cells slight toxicity was observed at pleconaril concentration of 10 μg/ml and at AG7088 concentration of 50 μg/ml. The median cytostatic concentration calculated as described above was 30.0 μg/ml for pleconaril and > 100 μg/ml for AG7088.

3.2. Antiviral assays

The median EC_{50} value determined by the microscopic CPE inhibition assay performed on five serotypes was 0.02 μg/ml (0.03 μM) (range, < 0.01–0.03 μg/ml) for AG7088 compared to 0.05 μg/ml (0.09 μM) (range, 0.03–0.07 μg/ml) for pleconaril ($P = 0.02$ for comparison between AG7088 and pleconaril). The median EC_{50} value determined by the spectrophotometric assay was 0.01 μg/ml (0.02 μM) (range < 0.01–0.08 μg/ml) for AG7088 and 0.05 μg/ml (0.08 μM) (range, 0.03–0.07 μg/ml) for pleconaril ($P = 0.15$).

Cytopathic effect inhibition assay was performed on 46 clinical HRV isolates originally recovered from nasopharyngeal washes of patients with common colds (Table 1). The median EC_{50} value determined microscopically was 0.01 μg/ml (0.02 μM) (range, < 0.01–0.04 μg/ml) for AG7088 compared to 0.07 μg/ml (0.17 μM) (range, < 0.01– > 1 μg/ml) for pleconaril ($P <$ 0.001 for comparison between AG7088 and pleconaril). The median EC_{50} value determined by spectrophotometric assay was 0.01 μg/ml (0.01 μM) (range, < 0.01–0.04 μg/ml) for AG7088 compared to 0.04 μg/ml (0.12 μM) (range, < 0.01– > 1 μg/ml) for pleconaril ($P < 0.001$). By either the microscopic or spectrophotometric methods the median EC_{50} value of AG7088 was < 1.0 μg/ml for all isolates and was > 1.0 μg/ml for ten of 46 (22%) isolates with pleconaril. We tested pleconaril at 10 μg/ml for seven of ten of these isolates. Inhibition was not observed in four of these seven isolates by both the microscopic and spectrophotometric methods.

The correlation of EC_{50} values obtained for all strains tested with the two methods, the microscopic and spectrophotometric assays, was high (r-value, 0.82; 95% CI, 0.74–0.88).

4. Discussion

Under these in vitro conditions AG7088 and pleconaril demonstrated comparable antiviral activity against five selected HRV serotypes. However, AG7088 appeared to be more potent and to have a broader antirhinoviral spectrum than pleconaril among clinical HRV isolates. At the concentrations tested, all 46 HRV clinical isolates were sensitive to AG7088 with the two assay end-points used, whereas the EC_{50} values for pleconaril were greater than 10 μg/ml in approximately 9% of isolates. The median EC_{50} values were also significantly different and depending on the method used, were 5–6-fold higher for pleconaril than for AG7088. These results suggest that a small subset of HRV strains circulating in the community lack susceptibility to pleconaril. However, a limitation of our conclusions is that we did not identify the serotypes of these different isolates and we did not performed further characterizations.

Any comparisons between AG7088 and pleconaril need to consider that these drugs have different antiviral mechanisms of action and have different pharmacokinetic characteristics. Pleconaril is an oral compound with good systemic distribution, whereas AG7088 is topically delivered. After a single oral dose of 200 mg or 5 mg/kg of pleconaril the maximum peak plasma or serum concentration of the drug in children was approximately 1.2 μg/ml, a value above EC_{50} values obtained for approximately 80% of clinical isolates tested (Abdel-Rahman and Kearns, 1998, 1999). In adults a single dose of 400 mg give peak plasma levels of 2–2.5 μg/ml (Abdel-Rahman and Kearns, 1999). The EC_{50} values for pleconaril were greater than 10 μg/ml in approximately 9% of isolates. Such plasma concentrations are not achievable with oral pleconaril but animal studies suggest that peak concentrations in nasal epithelium are several fold in excess of those observed in the plasma (Abdel-Rahman and Kearns, 1999). Of note, pleconaril shortens the illness duration in adults with acute upper respiratory tract infection (Hayden et al., 1999). The differences observed in

Table 1
In vitro antiviral effect of AG 7088 and pleconaril against clinical isolates of human rhinoviruses

HRV isolate	EC_{50} values (μg/ml)			
	Microscopic assay		Spectrophotometric assay	
	AG7088	Pleconaril	AG7088	Pleconaril
1	0.02	>10.0	0.02	>10.0
2	0.01	0.02	<0.01	0.02
3	0.02	0.02	0.02	0.02
4	0.01	0.08	0.03	0.14
5	0.03	0.08	0.03	0.10
6	0.02	0.10	0.02	0.03
7	0.02	0.22	0.01	0.13
8	0.04	0.17	0.02	0.10
9	0.01	0.22	<0.01	0.05
10	0.01	2.15	0.03	>10.0
11	0.03	0.03	0.01	0.01
12	<0.01	0.03	<0.01	0.02
13	0.01	0.02	0.02	0.03
14	0.02	0.11	0.01	0.17
15	0.01	<0.01	0.02	<0.01
16	<0.01	<0.01	<0.01	<0.01
17	<0.01	>1.0*	<0.01	>1.0*
18	<0.01	0.94	<0.01	0.05
19	<0.01	0.11	<0.01	0.14
20	<0.01	>1.0*	<0.01	>1.0*
21	<0.01	1.75	<0.01	0.54
22	<0.01	0.03	0.01	0.03
23	<0.01	<0.01	<0.01	0.01
24	0.02	0.09	0.02	0.10
25	0.02	0.22	0.02	0.04
26	0.01	0.10	<0.01	0.07
27	0.01	0.02	<0.01	<0.01
28	0.01	0.02	<0.01	<0.01
29	0.01	>1.0*	<0.01	>1.0*
30	0.02	>10.0	<0.01	>10.0
31	0.02	1.45	0.02	2.59
32	<0.01	0.03	<0.01	0.03
33	0.02	0.02	0.01	0.02
34	0.04	0.01	<0.01	0.03
35	<0.01	0.05	0.01	0.02
36	0.02	0.14	0.02	0.05
37	0.02	0.05	0.01	0.02
38	0.02	0.05	0.01	0.09
39	0.01	>10.0	<0.01	>10.0
40	0.01	0.01	<0.01	<0.01
41	0.02	0.05	0.04	0.07
42	0.02	0.02	0.01	0.01
43	0.02	0.05	0.02	0.04
44	0.02	0.05	<0.01	0.04
45	0.01	>10.0	0.01	>10.0
46	0.02	0.02	0.02	0.03
Median, range	0.01 (<0.01–0.04)	0.07 (<0.01–>1.0)	0.01 (<0.01–0.04)	0.04 (<0.01–>1.0)

* Not tested at concentration 10 μg/ml.

our in vitro study suggest that compared to AG7088, pleconaril potentially will not have activity against a small number of circulating rhinovirus strains. Whether this could translate in different clinical efficacy needs to be investigated in comparative clinical trials.

AG7088 is an irreversible peptidomimetic inhibitor of HRV 3C protease, which has a highly conserved active site (Werner et al., 1986; Skehan et al., 1990; Arruda et al., 1992, 1996; Rollins et al., 1993; Ohlin et al., 1994; Abdel-Rahman and Kearns, 1998, 1999; Hayden et al., 1999; Kearns et al., 1999; Patick et al., 1999). Our results showed that AG7088 is active against a wide range of HRV isolates suggesting that the affinity for the binding site is conserved among a wide range of viruses. A similar observation was done in a recent investigation showing that AG7088 was effective against 48 defined HRV serotypes with EC_{50} values comparable to those obtained in our study (Patick et al., 1999). Although these results suggest that primary resistance to this 3C protease inhibitor is uncommon, other RNA viruses such as human immunodeficiency viruses readily develops resistance when exposed to protease inhibitors. Although major differences exist between these two viral infections, whether resistant viruses could be selected by AG7088 treatment should be investigated in further investigations.

Both pleconaril and AG7088 are antiviral compounds effective against a wide range of HRV isolates. The clinical relevance of these in vitro results needs to be determined in controlled clinical trials.

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Phase II, Randomized, Double-Blind, Placebo-Controlled Studies of Ruprintrivir Nasal Spray 2-Percent Suspension for Prevention and Treatment of Experimentally Induced Rhinovirus Colds in Healthy Volunteers

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Human rhinovirus (HRV) infections are usually self-limited but may be associated with serious consequences, particularly in those with asthma and chronic respiratory disease. Effective antiviral agents are needed for preventing and treating HRV illnesses. Ruprintrivir (Agouron Pharmaceuticals, Inc., San Diego, Calif.) selectively inhibits HRV 3C protease and shows potent, broad-spectrum anti-HRV activity in vitro. We conducted three double-blind, placebo-controlled clinical trials in 202 healthy volunteers to assess the activity of ruprintrivir in experimental HRV infection. Subjects were randomized to receive intranasal ruprintrivir (8 mg) or placebo sprays as prophylaxis (two or five times daily [2×/day or 5×/day] for 5 days) starting 6 h before infection or as treatment (5×/day for 4 days) starting 24 h after infection. Ruprintrivir prophylaxis reduced the proportion of subjects with positive viral cultures (for 5×/day dosing groups, 44% for ruprintrivir treatment group versus 70% for placebo treatment group [$P = 0.03$]; for 2×/day dosing groups, 60% for ruprintrivir group versus 92% for placebo group [$P = 0.004$]) and viral titers but did not decrease the frequency of colds. Ruprintrivir treatment reduced the mean total daily symptom score (2.2 for ruprintrivir treatment group and 3.3 for the placebo treatment group [$P = 0.014$]) by 33%. Secondary endpoints, including viral titers, individual symptom scores, and nasal discharge weights, were also reduced by ruprintrivir treatment. Overall, ruprintrivir was well tolerated; blood-tinged mucus and nasal passage irritation were the most common adverse effects reported. Pharmacokinetic analysis of plasma and nasal ruprintrivir concentrations revealed intranasal drug residence with minimal systemic absorption. Results from these studies in experimental rhinoviral infection support continued investigation of intranasal ruprintrivir in the setting of natural HRV infection.

Human rhinoviruses (HRV) account for 40 to 50% of common colds on an annual basis and up to 80% of the colds during the autumn months in the Northern Hemisphere (2, 16). In healthy individuals, these infections are generally self-limiting and mild, although acute respiratory infections may be associated with substantial morbidity, loss of productivity, excess antibiotic use, and frequent self-medication with nonprescription remedies. HRV infection may also be complicated by acute sinusitis and otitis media and may cause exacerbations of asthma, chronic bronchitis, and cystic fibrosis, requiring acute care and hospital admission (7, 15, 20, 21, 24). For both otherwise healthy and high-risk individuals, antiviral treatment or prophylaxis would be desirable.

At this time, no antiviral agents are approved for the prevention or treatment of HRV infection. Several antiviral compounds with in vitro activity against HRV have been evaluated for the management of colds, including intranasal tremacamra, a soluble intercellular adhesion molecule 1 (ICAM-1); alpha interferon 2b; and the capsid binders, pirodavir and pleconaril (1, 8, 9–13, 22). While each of these investigational antiviral agents has important shortcomings, these studies have proven that prevention and early treatment of HRV colds are possible with antiviral compounds.

The HRV 3C protease is an enzyme responsible for the posttranslational cleavage of viral precursor polyproteins into their mature forms (19). Evaluation of the crystal structure of the HRV 3C protease has allowed the development of selective inhibitors targeting the enzyme's active site (17). Ruprintrivir (Agouron Pharmaceuticals, Inc., San Diego, Calif.) (formerly designated AG7088) is a potent, irreversible inhibitor of HRV 3C protease, developed through protein structure-based design methodologies. In vitro testing in cell protection assays has shown that ruprintrivir has a broad antipicornaviral spectrum, inhibiting the replication of all 48 HRV serotypes tested with a mean 50% effective concentration (EC_{50}) of 0.023 μM (range, 0.003 to 0.081 μM), as well as replication of other picornaviruses (18). Ruprintrivir also has been shown to inhibit HRV replication in transformed human bronchial epithelial cells (BEAS-2B), an effect associated with decreased production of proinflammatory cytokines interleukin-6 and interleukin-8, which may

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Exhibit B

have a role in the pathogenesis of rhinovirus symptoms (23, 25).

Ruprintrivir is a peptidomimetic compound (molecular weight, 598.7) with poor aqueous solubility and low oral bioavailability in animals (4). In healthy, uninfected volunteers, intranasal ruprintrivir spray was safe and well tolerated in doses of 4 mg or 8 mg 6×/day for 7 days (14). The present studies were designed to evaluate whether intranasal ruprintrivir could provide prophylactic and/or therapeutic benefit in experimentally infected volunteers.

MATERIALS AND METHODS

Subjects. Subjects were healthy volunteers, 18 to 60 years of age, who were selected on the basis of a serum neutralizing antibody titer of ≤1:2 to at least one of the two rhinovirus challenge strains used. In addition, subjects were free of symptoms of an upper respiratory tract infection during the 2 weeks prior to screening. Female subjects who were nonpregnant, nonlactating, and either of nonchildbearing potential or using acceptable methods of contraception were included. Urine pregnancy tests were performed at the beginning of the study. Subjects were excluded from the study for the following: recent history of asthma or history of chronic respiratory disease; history of significant medical or psychiatric illness; dysfunctional taste or olfaction; alcohol or substance abuse; use of topical nasal decongestants within 48 h prior to randomization or any other intranasal medication during the 2 weeks prior to study entry; use of an investigational drug within 30 days prior to randomization; and unwillingness to abstain from tobacco use throughout the study period. Subjects also were excluded if a screening examination demonstrated abnormal nasal mucosa or clinically significant deviated nasal septum. All subjects provided written, informed consent as approved by the institutional review board at each study site. This research was performed in compliance with all relevant federal guidelines and institutional policies.

Challenge viruses. The challenge viruses used for this study were obtained from safety-tested inoculum pools of rhinovirus (Hanks or HRV 39 strain) supplied by J. M. Gwaltney, Jr. (University of Virginia, Charlottesville). In a cytopathic inhibition assay using Hi-HeLa cells (18), the ruprintrivir EC_{50}s against HRV 39 and Hanks strains were determined to be 0.032 and <0.003 μM, respectively. On day 0, all subjects were inoculated with a targeted total of 100 to 300 50% tissue culture infectious doses ($TCID_{50}$) as nasal drops in a total volume of approximately 200 or 500 μl/nostril (site 3) of one of the two viruses. Subjects received the HRV challenge while in the supine position and were instructed to remain supine for 1 min after inoculation. The inoculation was repeated once at an interval of approximately 15 min. Based on back titration assays of the fresh inoculum pools performed at each site, the estimated delivered inocula were 30 $TCID_{50}$ of HRV 39 and 300 $TCID_{50}$ of HRV Hanks at study sites 1 and 2 and 10,000 $TCID_{50}$ of HRV 39 and 158 $TCID_{50}$ of HRV Hanks at study site 3.

Study medication. Ruprintrivir nasal spray (a 2% suspension) and placebo (vehicle) were supplied by Agouron Pharmaceuticals, Inc., in a USP type I amber glass vial fitted with an intranasal delivery device (Valois VP-7 nasal spray pump) intended to administer 100 μl of the formulation per spray actuation. Subjects received two sprays per nostril, alternating between nostrils, for a total estimated delivered dose of 8 mg per administration.

Study design. Ruprintrivir or placebo was administered starting either 6 h prior to viral challenge (prophylaxis) or 24 h after viral challenge (treatment). The ruprintrivir dosing regimen used was dependent upon the study site where the subjects were enrolled. Ruprintrivir prophylaxis was administered every 4 h while awake (five times daily [5×/day]) at site 3 (R. B. Turner, Medical University of South Carolina) or every 12 h (2×/day) at site 2 (F. G. Hayden, University of Virginia). Ruprintrivir treatment was administered 5×/day at sites 1 (J. M. Gwaltney, Jr., University of Virginia), 2, and 3. Subjects were housed at the study site, where they remained for the duration of the study period. For each trial, subjects were randomized in a 1:1 ratio to receive ruprintrivir or placebo for 4 days (treatment) or 5 days (prophylaxis). Randomization for the prophylaxis studies was stratified by viral strain. Randomization for the treatment study was stratified by study site and viral strain. Study personnel directly supervised study drug administration.

Assessments. Screening of all subjects was performed within 14 days of randomization and included a medical history and physical examination, nasal examination, rhinovirus serology (serum neutralizing antibody to specific challenge virus strains), clinical chemistry laboratory evaluations, complete blood count and hematology panel, and urinalysis. Laboratory assessments were also performed on days 0 and 5 (discharge) and at an exit evaluation 3 to 4 weeks after rhinovirus challenge. Safety and tolerability were assessed by observation and by volunteered reports of adverse effects, changes in physical examination, vital signs, nasal examinations, and routine hematology, chemistry, and urinalysis profiles.

Measurement of infection. Nasal washings for virologic analyses, including qualitative and quantitative viral cultures and HRV RNA quantification, were collected from subjects each morning on days 0 through 5. The initial isolate from each subject was subjected to neutralization testing with type-specific antisera to confirm its serotype. By using a standard method, nasal washings were cultured for rhinovirus on human embryonic lung fibroblast cells (WI-38 strain) purchased from commercial sources (3). Following adsorption, the inoculum was washed two times with phosphate-buffered saline and replenished with medium. Once-frozen (−70°C) aliquots of nasal wash samples that were positive in qualitative viral culture were thawed and subjected to quantitative viral titer determination by culture of serial 10-fold dilutions in duplicate monolayers. The titer of the virus in the original nasal lavage was calculated as the dilution in which viral growth was last seen in the quantitative assay. If one of the undiluted monolayer cultures was positive and the other was negative, the titer assigned was 0.7 $\log_{10}$ $TCID_{50}$/ml; if both were positive, the titer assigned was 1.2 $\log_{10}$ $TCID_{50}$/ml. Based on the volume of the inoculum, the limit of detection was calculated to be ≤0.4 $\log_{10}$ $TCID_{50}$/ml (for sites 1 and 2). If results on quantitative culture were negative, the results from the initial qualitative culture were reported. HRV RNA was quantified using the HRV-A TaqMan reverse transcription-PCR assay, which amplifies a conserved region of the 5′ untranslated region (G. Smith, S. Binford, P. Weady, and A. Patrick, unpublished results). Briefly, 560 μl of nasal lavage sample was extracted using a QIAamp viral RNA kit (Qiagen, Inc., Valencia, Calif.). The purified RNA was reverse transcribed using random hexamers followed by TaqMan PCR using a Prism 7700 instrument (Applied Biosystems, Foster City, Calif.).

Serum neutralizing antibody titers to the challenge virus were done at each site by standard methods (5). Serum specimens for antibody testing were collected during screening, immediately prior to virus challenge ´, and again 3 to 4 weeks later at the study exit visit (convalescent). Subjects with at least a fourfold increase in antibody titer to the challenge virus when the convalescent-phase serum sample was compared with the acute-phase serum sample were considered infected.

Measurement of illness. The presence and severity of eight symptoms (sneezing, malaise, rhinorrhea, sore throat, headache, chilliness, nasal obstruction or congestion, and cough) were assessed by subjects on day 0 prior to viral challenge and then twice daily, once in the morning prior to nasal washing and again in the evening prior to the 7:00 p.m. dose of study drug. The severity of each symptom was rated on a five-point scale, ranging from 0 (none) to 4 (very severe). Subjects were instructed to score each symptom on the basis of the maximal severity experienced since the previous report. The sum of daily scores for all eight symptoms comprised the total daily symptom score; the sum of scores for sneezing, rhinorrhea, sore throat, nasal obstruction or congestion, and cough comprised the total daily respiratory symptom score. Nasal discharge weights were determined daily on days 0 to 4 by having subjects collect all tissues used for nose blowing.

Pharmacokinetics. Samples were collected to determine drug residence in the nasal cavity and the extent of systemic exposure. Collection of nasal washes was performed on day 2 within 15 min prior to the fourth ruprintrivir dose for 5×/day dosing groups or 15 min prior to and 6 h after the first dose for the 2×/day dosing group. On day 3, blood samples were collected 15 min prior to and 1, 2, and 4 h following the third dose of ruprintrivir in 5×/day dosing groups and 15 min prior to and 1, 4, 8, and 12 h after the dose in the 2×/day dosing group.

Concentrations of ruprintrivir and its primary metabolite AG7185 in plasma and nasal washings were determined using TurbulonSpray liquid chromatography with tandem mass spectrometric detection (Alta Analytical Laboratory, El Dorado Hills, Calif.). The lower limits of detection of this assay were 0.2 ng/ml for plasma samples and 1.0 ng/ml for nasal wash samples. The following pharmacokinetic parameters were determined from the concentrations of ruprintrivir and AG7185 in plasma: maximum concentration of drug in plasma (C_{max}), time to maximum concentration of drug in plasma (T_{max}), and area under the concentration-versus-time curve (AUC) during the dosing interval. C_{max} and T_{max} estimates were obtained from the plasma drug concentration-versus-time curve. AUC was calculated using the log linear trapezoidal rule. Concentrations of ruprintrivir and AG7185 were measured in nasal washes from each nostril and were summed to obtain the amount of ruprintrivir and AG7185 recovered from the nose.

TABLE 1. Demographic and baseline characteristics of the subjects by treatment group

Characteristic	Value for treatment group					
	5×/day prophylaxis		2×/day prophylaxis		5×/day treatment	
	Placebo (n = 27)	Ruprintrivir (n = 25)	Placebo (n = 25)	Ruprintrivir (n = 26)	Placebo (n = 49)	Ruprintrivir (n = 50)
Age (yr)						
Mean (SD)	32.7 (10.4)	31.5 (9.9)	21.2 (4.1)	21.2 (3.1)	22.3 (5.7)	23.3 (6.7)
Range	18–58	20–54	18–38	18–31	18–44	18–43
Sex (n [%])						
Women	15 (56)	17 (68)	17 (68)	18 (69)	29 (59)	23 (46)
Men	12 (44)	8 (32)	8 (32)	8 (31)	20 (41)	27 (54)
Race (n [%])						
White	20 (74)	18 (72)	19 (76)	19 (73)	39 (80)	45 (90)
Black	5 (19)	6 (24)	2 (8)	3 (12)	6 (12)	1 (2)
Asian	2 (7)	1 (4)	0	2 (8)	2 (4)	3 (6)
Hispanic	0	0	2 (8)	1 (4)	1 (2)	0
Other	0	0	2 (8)	1 (4)	1 (2)	1 (2)
Virus strain						
Hanks	22	21	25	26	17	18
HRV 39	5	4	0	0	32	32
History of allergy (n [%])	3 (11)	7 (28)	10 (40)	5 (19)	11 (22)	14 (28)

Study endpoints. The primary efficacy measure for prophylaxis was defined prospectively as a reduction in the proportion of subjects with positive viral culture due to the rhinovirus challenge strain. The primary efficacy measure for treatment was defined by a reduction in the total mean symptom score for days 1 through 4 for subjects who became infected with the challenge virus. Infection was defined by a positive culture, fourfold or greater increase in virus-specific serum neutralizing antibody titers, or both. Secondary efficacy endpoints included the incidence of clinical colds, change in viral titer over time, mean total and respiratory symptom scores (for prophylaxis), change in individual symptom scores over time, and nasal discharge weights. The presence of a clinical cold was determined using the previously described modified Jackson criteria (6). For each subject, the area under the $\log_{10}$ viral titer-versus-time curve was computed. In a separate analysis, the change in viral RNA level over time was also determined for infected subjects.

Data analysis. For the primary and secondary efficacy endpoints, both intent-to-treat and efficacy-evaluable analyses were performed. However, since the results were nearly identical, only efficacy-evaluable results are presented here. For the prophylaxis studies, evaluable subjects had a negative nasal washing culture prior to viral inoculation and completed all study medication and procedures through day 5. For the treatment study, evaluable subjects included those who had a negative nasal culture prior to viral challenge, completed all study medication and procedures through day 5, and had evidence of infection with the challenge virus strain. The safety analysis included all subjects who received study drug. The null hypothesis in the prophylaxis study that the percentage of subjects experiencing positive culture in the ruprintrivir group was equal to that in the placebo group was tested using a chi-square test (2×/day; no stratification) or Cochran-Mantel-Haenszel test (5×/day; stratified by virus strain), as appropriate. In the treatment study, mean total symptom scores for days 1 through 4 were compared using analysis of variance (ANOVA) or analysis of covariance (ANCOVA) to test the null hypothesis that the mean total symptom score in the ruprintrivir treatment group was equal to that of the placebo group. The ANCOVA model for this dosing group included effects for treatment, study site, and challenge virus strain and was adjusted for baseline values. The baseline value was the last value reported after the virus challenge and before the first treatment dose. The frequencies of antibody response, infection, and clinical cold were summarized by treatment and analyzed by the chi-square test or Cochran-Mantel-Haenszel test, as appropriate. Mean respiratory symptom scores for days 1 through 4, daily total symptom scores, daily and cumulative nasal discharge weights, area under the $\log_{10}$ viral titer-versus-time curve, and $\log_{10}$ viral titer and viral RNA by day were summarized by treatment and compared using ANOVA or ANCOVA, as appropriate.

In this proof-of-principle study, all P values reported are one sided, and a one-sided P value of <0.05 was considered to be statistically significant. All statistical analyses were performed using SAS software version 6.12 (SAS Institute, Inc., Cary, N.C.).

RESULTS

Subjects. A total of 202 subjects (101 subjects each in the ruprintrivir and placebo groups) were enrolled in these studies. All randomized subjects completed study drug administration, and safety was evaluated in all subjects. Of the 202 subjects enrolled, 194 could be evaluated for efficacy (see below).

The demographic characteristics of the subjects, including age, sex, and race, are summarized by treatment group in Table 1. The age of subjects at sites 1 and 2 (Charlottesville, Va.) were, on average, 10 years lower than at site 3 (Medical University of South Carolina), reflecting an increased use of student volunteers at the Charlottesville sites. A history of allergy was reported in 7 (28%) subjects in the 5×/day prophylaxis study, 5 (19%) subjects in 2×/day prophylaxis study, and 14 (28%) subjects in the 5×/day treatment study receiving ruprintrivir and in 24 (24%) of all subjects receiving placebo.

Prophylactic efficacy. All subjects in the 5×/day study were evaluated for efficacy. One subject in the 2×/day study (ruprintrivir arm) was excluded from efficacy analyses due to a positive culture prior to virus inoculation. Table 2 summarizes primary and secondary measures of efficacy for evaluable subjects in the two prophylaxis studies. In both groups, the proportion of subjects with one or more positive viral cultures was significantly reduced by treatment with ruprintrivir compared with treatment with placebo. In the 5×/day study, this frequency was reduced by 37%, and in the 2×/day study, it was reduced by 35%. In both prophylaxis studies, subjects receiving ruprintrivir demonstrated significantly lower viral titers and RNA levels than subjects receiving placebo (Fig. 1 and 2). Since the quantification of RNA is not sensitive to the presence of ruprintrivir, the correlation between these two independent measurements suggests that drug carryover did not confound the infectious viral titer results obtained. Analyses of area under the $\log_{10}$ viral titer-versus-time curve revealed a significantly lower AUC in subjects receiving ruprintrivir versus placebo (Table 2). Of note, much higher copy numbers of RNA (ap-

TABLE 2. Prophylactic antiviral efficacy of ruprintrivir in experimental rhinovirus infection

Characteristic	Value for treatment group					
	5×/day			2×/day		
	Ruprintrivir (n = 25)	Placebo (n = 27)	P value[f]	Ruprintrivir (n = 25)	Placebo (n = 25)	P value[f]
No. (%) with positive viral culture [95% CI][a]	11 (44) [27, 63]	19 (70) [52, 84]	0.03	15 (60) [41, 77]	23 (92) [75, 98]	0.004
No. (%) with antibody response[b] [95% CI]	9 (36) [20, 55]	9 (33) [19, 52]	0.60	18 (72) [52, 86]	20 (80) [61, 91]	0.25
No. (%) infected[c] [95% CI]	14 (56) [37, 73]	21 (78) [59, 89]	0.05	20 (80) [61, 91]	23 (92) [75, 98]	0.11
No. (%) with cold[d] [95% CI]	6 (43) [21, 67]	11 (52) [32, 72]	0.24	10 (50) [30, 70]	13 (57) [37, 74]	0.33
Mean AUC normalized by day (log_{10} TCID · day/ml) (SD)	0.74 (0.1)	1.4 (0.8)	<0.001	0.91 (0.3)	1.07 (0.3)	0.03
Mean daily total symptom score/day (SD)	2.4 (2.6)	3.5 (3.9)	0.12	1.9 (2.0)	2.4 (2.2)	0.18
Mean daily respiratory symptom score/day[e] (SD)	1.9 (2.4)	2.8 (3.1)	0.12	1.5 (1.5)	1.9 (1.7)	0.18
Mean cumulative nasal discharge weight (g) (days 0–4) (SD)	11.3 (14.3)	24.9 (57.4)	0.12	16.4 (24.7)	28.0 (23.7)	0.05

[a] 95% confidence intervals (95% CI) are shown as percentages in brackets.
[b] Defined as at least a fourfold rise in antibody titer to the challenge virus.
[c] Subjects with positive viral culture and/or antibody response.
[d] Number of colds in those infected, defined by modified Jackson criteria.
[e] Includes sneezing, rhinorrhea, sore throat, nasal obstruction or congestion, and cough.
[f] P values comparing values for ruprintrivir-treated subjects to placebo-treated subjects. All P values are one sided; a one-sided P value of <0.05 was considered to be statistically significant.

proximately 5 to 6 log_{10} units at peak) were detected than by infectious virus titers (approximately 2 log_{10} units at peak), but the patterns were similar over time.

No important differences were observed between ruprintrivir and placebo treatment groups with regard to rates of seroconversion or clinical colds, although the overall infection rate was reduced by 28% in the 5×/day ruprintrivir treatment group compared to the rate for the placebo treatment group.

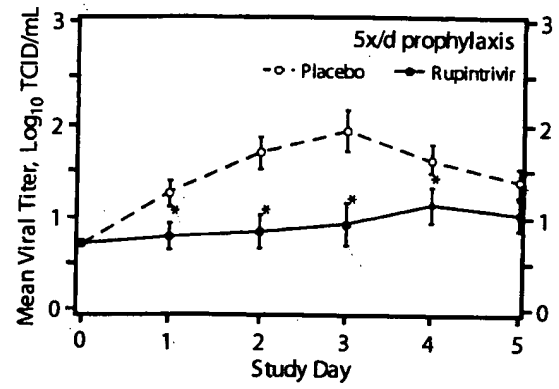
Total symptom scores by day (Fig. 3) tended to be lower in ruprintrivir-treated subjects in both prophylactic trials. In addition, total respiratory symptom scores (which included sneezing, rhinorrhea, sore throat, nasal obstruction or congestion, and cough) tended to be lower in ruprintrivir-treated subjects than in placebo-treated subjects (Table 2). Overall, nasal discharge weights per day (Fig. 4) were lower for ruprintrivir-treated subjects than for placebo-treated subjects and significantly reduced at day 2 to 3 in the 5×/day group and at day 1 to 2 and day 2 to 3 in the 2×/day group. Cumulative (i.e., total across time) nasal discharge weights were reduced by 55% in 5×/day and 41% in 2×/day ruprintrivir prophylaxis groups, but the difference between groups was significant only for subjects in the 2×/day prophylactic study (Table 2).

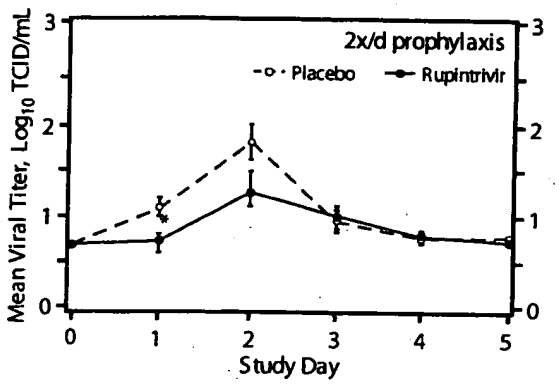
Treatment efficacy. In the treatment study, six ruprintrivir-treated subjects and one placebo-treated subject were excluded from the efficacy evaluation due to no evidence of infection or a positive culture prior to virus challenge, respectively. In this study, 39 of 44 (89%) infected subjects receiving ruprintrivir and 45 of 48 (94%) infected subjects receiving placebo shed challenge virus (Table 3). For these subjects, all 44 (100%) subjects receiving ruprintrivir and all 48 (100%) subjects receiving placebo tested positive for viral RNA by the HRV-A TaqMan RT-PCR assay.

In these subjects, the frequency of illness was not reduced by ruprintrivir. However, the severity of illness was significantly lower in ruprintrivir recipients than in those receiving placebo. The mean daily total symptom score over the 4 treatment days was 33% lower in ruprintrivir-treated subjects than that of placebo-treated subjects (2.2 versus 3.3, respectively [P = 0.01]). Similarly, total symptom scores by day of treatment were lower in ruprintrivir-treated subjects on days 2 and 3 ($P <$ 0.05) (Fig. 3). Significantly lower scores for the individual symptoms of sneezing, rhinorrhea, and nasal obstruction or congestion were observed in ruprintrivir-treated subjects compared with placebo-treated subjects on days 1 to 3, 2 or 3, and 2 to 4, respectively (Fig. 5). In addition, ruprintrivir treatment significantly lowered nasal discharge weights during days 2 or 3, 3 or 4, and 4 or 5 (Fig. 4), and cumulative nasal discharge weights measured over the 4 days of treatment were 40% lower (Table 3). Subjects receiving ruprintrivir also demonstrated significantly lower viral titers and RNA levels than placebo-treated subjects on days 2, 3, and 5 and on days 2 and 3, respectively (Fig. 1 and 2).

Tolerability. There were no serious adverse effects reported, and there were not any withdrawals due to the development of adverse effects. Overall, 58 of 202 evaluable subjects experienced treatment-emergent adverse effects. Of these 58 subjects, 19 reported local effects, including blood-tinged mucus (n = 11), nasal passage irritation (n = 3), sinus pain (n = 3), postnasal drip (n = 2), and nasal dryness (n = 1). (Note that a subject could report more than one adverse effect.) Local nasal effects were more common in subjects receiving 5×/day dosing (prophylaxis, 19%; treatment, 8%) than in subjects receiving 2×/day prophylaxis (2%). Local nasal effects were slightly more common in ruprintrivir-treated subjects than placebo-treated subjects (11% versus 8%).

Adverse events reported by ≥5% of subjects are summarized by study (prophylaxis or treatment study) in Table 4. In the 5×/day prophylaxis study, adverse events included blood-





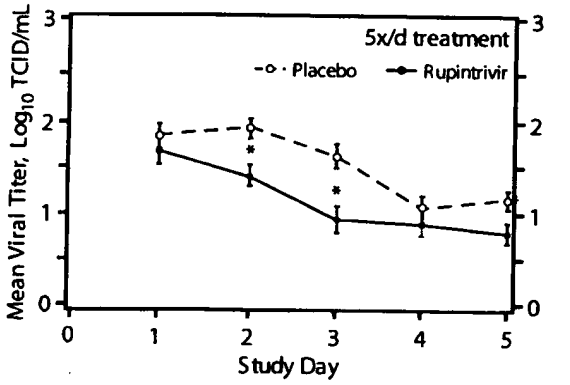
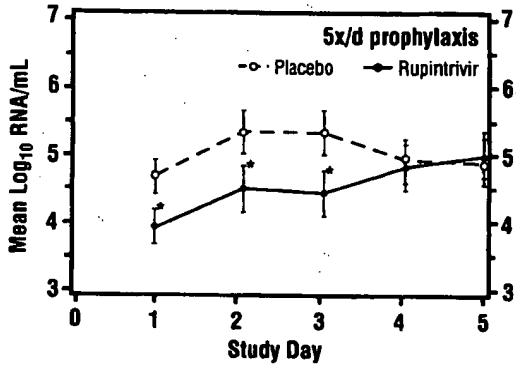
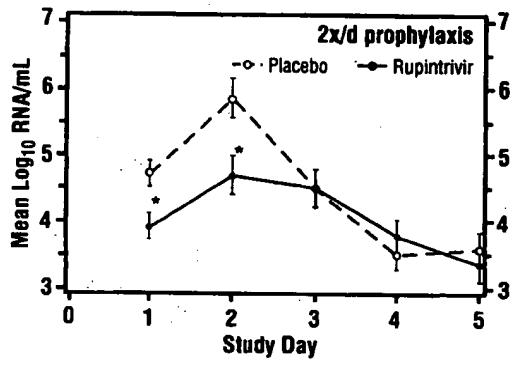


FIG. 1. Mean $\log_{10}$ viral titer in nasal lavage fluid over time for subjects for whom efficacy could be evaluated (5×/day [5x/d] prophylaxis, 2×/day prophylaxis, and 5×/day treatment groups). Values are given as means ± standard errors (error bars) by the least-square method. Values for the ruprintrivir-treated subjects that were significantly different (one-sided P values of <0.05) from the values for the placebo-treated subjects by ANOVA with effects for treatment (all groups), site (5x/d treatment group), and challenge virus strain (5x/d prophylaxis and treatment groups) and by ANCOVA with effects for treatment, study site, and challenge virus strain adjusted for baseline values (days 2 to 5 of 5x/d treatment group) are indicated by asterisks.





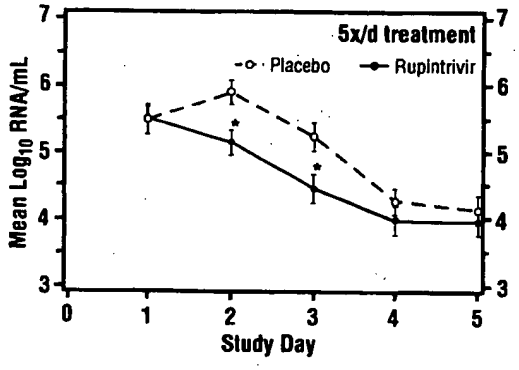


FIG. 2. Mean $\log_{10}$ viral RNA/ml in nasal lavage fluid over time for subjects for whom efficacy could be evaluated (5×/day [5x/d] prophylaxis, 2×/day prophylaxis, and 5×/day treatment groups). Values are given as means ± standard errors (error bars) by the least-square method. Values for the ruprintrivir-treated subjects that were significantly different (one-sided P values of <0.05) from the values for the placebo-treated subjects by ANOVA with effects for treatment (all groups), site (5x/d treatment group), and challenge virus strain (5x/d prophylaxis and treatment groups) and by ANCOVA with effects for treatment, study site, and challenge virus strain adjusted for baseline values (days 2 to 5 of 5x/d treatment group) are indicated by asterisks.

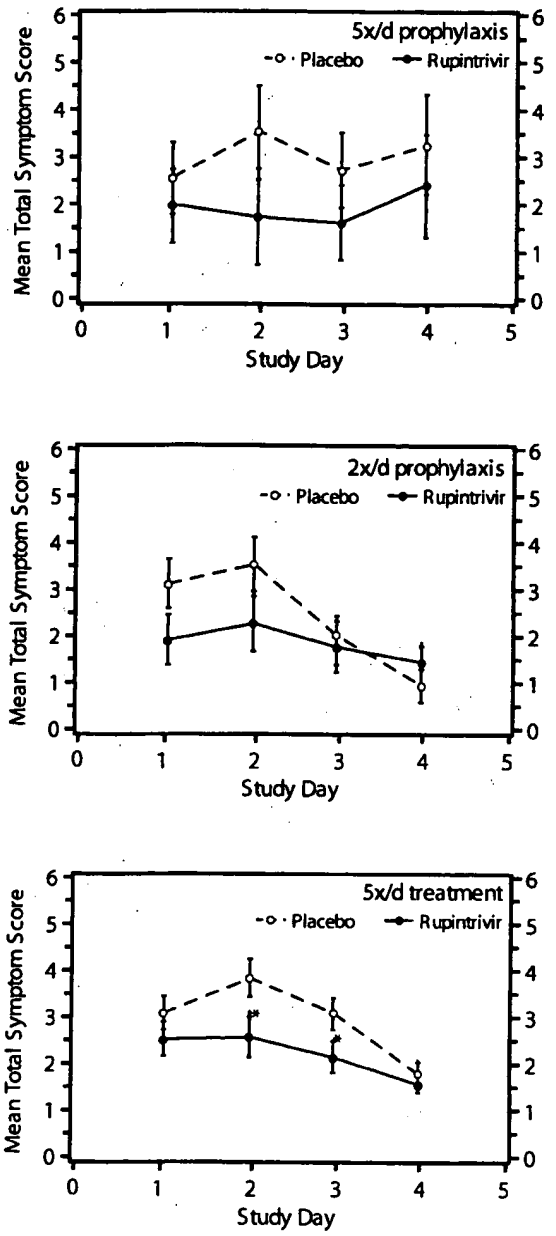


FIG. 3. Mean total symptom score over time for subjects for whom efficacy could be evaluated (5×/day [5x/d] prophylaxis, 2×/day prophylaxis, and 5×/day treatment groups). For both treatment and prophylaxis groups, baseline values were virtually 0. Values are given as means ± standard errors (error bars) by the least-square method. Values for the ruprintrivir-treated subjects that were significantly different (one-sided P values of <0.05) from the values for the placebo-treated subjects by ANOVA for treatment (5×/day prophylaxis and 2×/day prophylaxis groups) and challenge virus strain (5×/day prophylaxis group) and by ANCOVA with effects for treatment, study site, challenge virus strain adjusted for baseline values are indicated by asterisks.

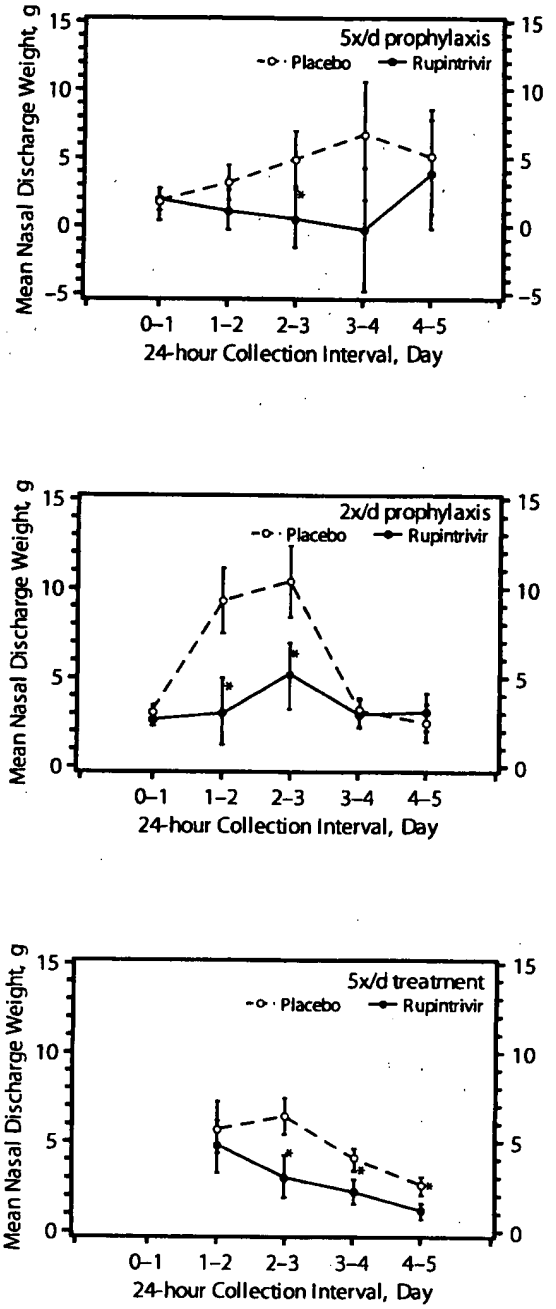


FIG. 4. Mean nasal discharge weight over time for subjects in whom efficacy could be evaluated (5×/day [5x/d] prophylaxis, 2×/day prophylaxis, and 5×/day treatment groups). Values are given as means ± standard errors (error bars) by the least-square method. Values for the ruprintrivir-treated subjects that were significantly different (one-sided P values of <0.05) from the values for the placebo-treated subjects by ANOVA with effects for treatment (all treatment groups), study site (5x/d treatment group), and challenge virus strain (5x/d prophylaxis and treatment groups) are indicated by asterisks.

tinged mucus (11% for placebo-treated subjects versus 16% for ruprintrivir-treated subjects) and nasal passage irritation (7% for placebo-treated subjects versus 4% for ruprintrivir-treated subjects). In the 2×/day prophylaxis study, no individual adverse event was reported by >5% of subjects receiving either ruprintrivir or placebo. In the 5×/day treatment study, adverse events occurring in >5% of subjects included ear disorder (4% for placebo-treated subjects versus 8% for ruprintrivir-treated

TABLE 3. Effects of intranasal ruprintrivir on virologic and illness measures in subjects receiving early treatment for experimental rhinovirus infection

Characteristic	Value for treatment group		
	Ruprintrivir (n = 44)	Placebo (n = 48)	P value[e]
No. (%) with positive viral culture [95% CI][a]	39 (89) [76, 95]	45 (94) [83, 98]	0.19
No. (%) with antibody response[b] [95% CI]	21 (48) [34, 62]	26 (54) [40, 67]	0.29
No. (%) infected[c]	44 (100)	48 (100)	NA
No. (%) with cold[d] [95% CI]	23 (52) [38, 66]	30 (63) [48, 75]	0.14
Mean AUC normalized by day (log_{10} TCID · day/ml) (SD)	1.1 (0.5)	1.6 (0.8)	<0.001
Mean daily total symptom score/day (SD)	2.2 (2.4)	3.3 (2.5)	0.01
Mean daily respiratory symptom score/day[e] (SD)	1.9 (1.7)	2.8 (2.0)	0.007
Mean cumulative nasal discharge weight (g) (days 0–4) (SD)	11.1 (14.6)	18.5 (18.1)	0.01

[a] 95% confidence intervals (95% CI) are shown as percentages in brackets.
[b] Defined as at least a fourfold rise in antibody titer to the challenge virus.
[c] Subjects with positive viral culture and/or antibody response.
[d] Number of colds in those infected, defined by modified Jackson criteria.
[e] Includes sneezing, rhinorrhea, sore throat, nasal obstruction or congestion, and cough. P values comparing values for ruprintrivir-treated subjects to placebo-treated subjects. All P values are one sided; a one-sided P value of <0.05 was considered to be statistically significant. NA, not available.

subjects), blood-tinged mucus (2% for placebo-treated subjects versus 6% for ruprintrivir-treated subjects), and nausea (2% for placebo-treated subjects versus 6% for ruprintrivir-treated subjects). Ear disorder was defined as stuffy, clogged, or popping ears and was considered related to HRV infection.

The only clinically relevant changes in physical examination were tonsillar exudate and cervical adenopathy in one placebo-treated subject on day 4 of the study. No clinically significant differences were observed between ruprintrivir- and placebo-treated subjects regarding vital signs, day 5 nasal examination, hematology, and clinical chemistry laboratory evaluations, and urinalysis findings (data not shown). Urinalysis detected 16 subjects (6 ruprintrivir-treated and 10 placebo-treated subjects) with trace proteinuria or with a score of 1+ for proteinuria on day 5; this resolved in all four cases for which a repeat urinalysis was performed.

Pharmacokinetics. The concentrations of ruprintrivir and its metabolite AG7185 in plasma were determined on day 3 of dosing. Nonmeasurable plasma ruprintrivir concentrations were observed in 63% of ruprintrivir-treated subjects; in the remainder, ruprintrivir concentrations were low (≤0.92 ng/ml). Plasma ruprintrivir AUC values ranged from 0.12 to 3.04, 0 to 1.41, and 0.11 to 1.66 ng · h/ml for 5×/day prophylaxis, 2×/day prophylaxis, and 5×/day treatment groups, respectively, confirming the minimal systemic exposure associated with multiple doses of ruprintrivir. Measurable concentrations of AG7185 were observed in all ruprintrivir-treated subjects; all AG7185 concentrations were ≤12.18 ng/ml.

To determine ruprintrivir residence in the nasal cavity following intranasal administration, nasal washes were collected from a subset of subjects on day 2 of dosing prior to the fourth dose (5×/day dosing) or prior to and 6 h following the first dose (2×/day dosing). Prior to the fourth dose, the amount of ruprintrivir recovered from the nasal washings varied substantially, ranging from 2.6 to 59.8 μg in the 5×/day prophylaxis group and from 0.005 to 62.7 μg in the 5×/day treatment group. In the 2×/day prophylaxis group, the amount of ruprintrivir recovered also varied, with 0 to 1.05 μg predose to 0 to 26.2 μg at 6 h after dosing. Drug concentrations in nasal washings were not corrected for dilution factors.

DISCUSSION

In this series of proof-of-principle studies, we have shown that intranasal administration of the novel rhinovirus 3C protease inhibitor ruprintrivir was well tolerated and provided significant antiviral effects. Specifically, ruprintrivir was effective in reducing the incidence of positive viral culture and measures of quantitative viral replication when used prophylactically or therapeutically and in attenuating symptom severity when used for early treatment. Although none of the studies showed significant reductions in the frequency of clinical colds, virologic and clinical secondary endpoints demonstrated trends favoring ruprintrivir over placebo administration. While the prophylaxis studies did not have the power to detect differences in secondary endpoints, data supporting the antiviral efficacy of ruprintrivir were observed, particularly reductions in viral titers over time, RNA levels, and daily nasal discharge weights.

The possibilities that reductions in viral titer observed for ruprintrivir-treated subjects might have been influenced by the inhibitory effect of residual ruprintrivir in the nasal wash (i.e., drug carryover) and that ruprintrivir may have interfered with viral replication, leading to false-negative viral cultures, cannot be excluded. Because this 3C protease inhibitor does not affect early events (e.g., attachment and penetration) but rather blocks a late step in replication, removal of drug from inoculated monolayers by washing after the adsorption period should be an effective means of avoiding carryover effects. Indeed, the correspondence between viral RNA levels (whose quantification is not sensitive to ruprintrivir) and viral titers indicate that drug carryover was not a confounding factor in the current studies.

As in previous studies, intranasal ruprintrivir was well tolerated. Study drug-related adverse events were generally mild in severity and local in nature (blood-tinged mucus and nasal irritation). Subjects who received the study drug 2×/day rather than 5×/day experienced fewer study drug-related adverse events, including blood-tinged mucus and nasal passage irritation. This observation is consistent with the conclusion that mucosal irritation related to repetitive nasal spray administra-

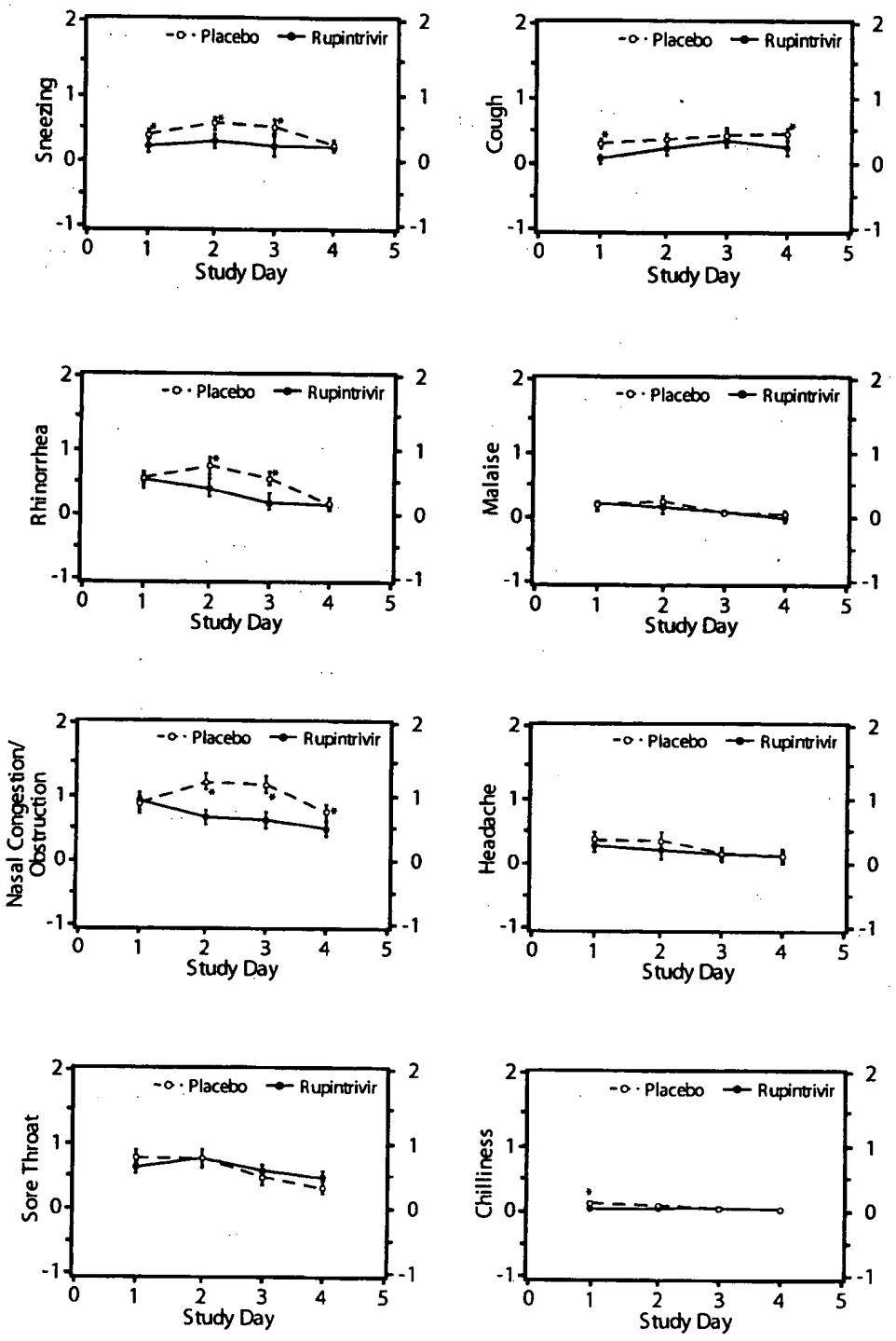


FIG. 5. Mean scores for individual symptoms (sneezing, cough, rhinorrhea, malaise, nasal congestion or obstruction, headache, sore throat, and chilliness) over time for subjects in whom efficacy could be evaluated (5×/day treatment group). Symptom scores before challenge were 0 for all subjects, except for one subject who had a baseline rhinorrhea score of 1. Values are given as means ± standard errors (error bars) by the least-square method. Values for the ruprintrivir-treated subjects that were significantly different (one-sided P values of <0.05) from the values for the placebo-treated subjects by ANCOVA with effects for treatment, study site, and challenge virus strain adjusted for baseline values are indicated by asterisks.

TABLE 4. Adverse effects reported in ≥5% of subjects receiving ruprintrivir or placebo for experimental rhinovirus infection

Adverse effect	No. (%) of subjects in treatment group					
	5×/day prophylaxis		2×/day prophylaxis		5×/day treatment	
	Placebo (n = 27)	Ruprintrivir (n = 25)	Placebo (n = 25)	Ruprintrivir (n = 26)	Placebo (n = 49)	Ruprintrivir (n = 50)
Subjects with adverse events[a]	10 (37)	10 (40)	5 (20)	4 (15)	12 (24)	17 (34)
Blood-tinged mucus	3 (11)	4 (16)	0	0	1 (2)	3 (6)
Nasal passage irritation	2 (7)	1 (4)	0	0	0	0
Ear disorder[b]	0	0	0	0	2 (4)	4 (8)
Nausea	1 (4)	0	0	0	1 (2)	3 (6)

[a] Subjects who experienced more than one adverse event were counted once.
[b] Stuffy, clogged, or popping ears.

tion, rather than direct drug effect, was the likely cause for the adverse events noted.

Following intranasal administration of ruprintrivir, low concentrations of ruprintrivir and its metabolite AG7185 in plasma were observed, a finding that suggests that systemic exposure is minimal following multiple doses of drug and is consistent with the lack of systemic adverse events observed. Plasma AG7185 concentrations were higher than those observed in phase I studies; this result may be attributed to the differences in populations studied. In phase I studies, healthy volunteers had normal mucosa, whereas rhinovirus-infected volunteers in the present studies might have had some degree of loss of mucosal integrity due to infectious rhinitis. Significant amounts of ruprintrivir were recovered from the nose on day 2 of study in all dosing groups. While this is of particular interest in subjects given only two doses a day, the relationship between detectable drug levels in nasal washings and antiviral effect remains unclear. It is possible that alternative formulations that enhance delivery of ruprintrivir to the nasal epithelium or promote prolonged drug retention at higher levels may provide greater antiviral effects.

It is instructive to consider the results of these studies in the context of earlier trials conducted with intranasal antirhinovirus compounds having different mechanisms of antiviral action. The magnitude of the clinical effects observed in this study of early treatment (24 h postinoculation) is broadly comparable to that seen with intranasal administration of tremacamra, a soluble ICAM-1 receptor decoy, initiated at 4 h before or 12 h after infection and greater than those observed with administration of intranasal alpha interferon 2b or the capsid-binding agent pirodavir at 24 h after inoculation (9, 11, 22). Early intranasal tremacamra spray administration reduced cold frequency, symptom scores, and nasal mucus weights by 23, 45, and 56%, respectively, compared to treatment with placebo, and treatment with interferon drops reduced the values by 2, 20, and 52%, respectively; pirodavir did not affect these endpoints. Of note, neither alpha interferon 2b nor pirodavir alone was found to be therapeutically effective in treating natural rhinovirus colds, and it is likely that combinations of antiviral agents and antimediator drugs will be needed to provide maximal therapeutic benefit (8, 12, 13). With respect to prophylactic activity, intranasal administration of various interferons and pirodavir (6×/day, but not 3×/day) has been shown to reduce the frequency of experimental colds, and intranasal alpha interferon 2b reduces the risk of natural rhinovirus illness (1, 9). In contrast, intranasal ruprintrivir exerted significant antiviral effects but did not diminish the frequency of experimental colds. This finding is unexpected, and the explanation for it is unclear.

Ruprintrivir is formulated as a suspension intended for intranasal delivery directly to the site of viral replication and represents the first protease inhibitor to be evaluated clinically for in vivo activity against HRV infection. The broad antipicornavirus spectrum of ruprintrivir, combined with its antiviral activity and safety in these studies of experimental rhinovirus infection, indicate that it warrants further testing for the management of natural rhinovirus infections. To effectively inhibit the inflammatory cascade of the common cold, ruprintrivir treatment will need to be initiated rapidly after cold symptoms are recognized. This may be the greatest challenge for clinicians in the implementation of treatment with ruprintrivir or other antirhinoviral agents.

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